Inhibitory effect of Lactic Acid Bacteria isolated from minced beef meat on some pathogenic bacteria

May A.H. AL-Allaf[1] , Amera M.M. Al-Rawi[2] , Alaa T. Al-Mola[2]

[1] *Environmental & Pollution Control Research Center, Mosul University , Mosul , Iraq*

E-mail : Mayallaf2@yahoo.com

[2] *Department of Biology , College of Science , Mosul University , Mosul , Iraq*

(**Received:** 11 / 10 / 2009 ---- **Accepted:** 27 / 4 / 2010)

Abstract

The aim of this research was to study the effect of the cell free culture of Lactic Acid Bacteria (LAB) as an antimicrobial feature to inhibit growth of some gram positive and gram negative bacteria associated of meat spoilage. Isolation and identification of LAB from minced beef meat by using de Man Rogosa and Sharpe agar (MRS) medium was carried out . Antimicrobial activities were measured by using the agar well diffusion method (Muller Hinton Agar) on some Gram positive and Gram negative pathogenic bacteria which involved (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*) were obtained by the agar diffusion method using

Results showed that the maximum inhibition zone (4mm) was detected against *Bacillus cereus* followed by *Proteus spp.*, *Staph. aureus*, *Pseudomonas aerugenosa*, *Streptococcus pneumoniae*, *E. coli*, *Klebsiella pneumoniae* , *Salmonella* spp. , *Corynebacterium* spp. Which were (2.8, 2.5, 2, 1.8, 1.8, 1, 0.75, 0.6 mm) respectively after 24 hours incubation period .

Introduction

Meat is a the major source of protein and valuable qualities of vitamins for most people in many parts of the world , thus they are essential for the growth, repair and maintenance of body cells and necessary for our everyday activities[(1)]. Due to the chemical composition and biological characteristics, meats are highly perishable foods which provide excellent source for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss[(2)]. The most important bacterial spoilage of meat was caused by lactic acid bacteria which are physiologically related group of fastidious and ubiquitous gram positive organisms includes many species such as *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* [(3)]. They are widely used for fermentation and preservation of wide range of meat, milk and vegetable foods [(4)] for extending the shelf life and improving the hygienic quality of various fermented products[(5)] that contain lactic acid bacteria. The preservative activity of these bacteria was due to their ability to produce a variety of antimicrobial substances such as ethanol, formic acid, acetone, hydrogen peroxide, diacetyl and bacteriocins[(6)]. The antimicrobial spectrum against competitor natural flora was frequently includes spoilage bacteria and food-borne pathogens such as *L. monocytogenes* and *S. aureus* [(7,8)].

Limited studies were carried out about the important role of bacteriocins for preservation and extension of minced meat by using LAB as a biopreservation in Mosul city as a native work.

Materials and Methods

Isolation of LAB

Samples of minced meat were collected randomly from different butchers shop in Mosul city, these samples were transported to the laboratory immediately using cool box (4°C) and tested directly.

LAB were isolated from minced meat by adding 10gm meat sample and mixed with 90 ml of normal saline solution (8.5gm NaCl / L) and homogenizing for 2 minute[(9)]. Serial dilutions up to 10^{-7} were prepared and appropriate dilutions were plated onto de Man Rogosa and Sharpe agar (HiMedia Laboratories Pvt. India)[(10)]. Duplicate plates were incubated at 37 °C for 24 hours , after growing a single colony was tested and examinated morphologically and microscopically for purity, then the bacteria was identified depending on biochemical tests including production of catalase enzyme and indol test as well as motility , after that subculture in de Man Rogosa and Sharpe broth (MRS broth) [(11,12)] .

Preparation of Cell-Free Filtrate

Tube containing MRS broth medium was inoculated with 1% of fresh culture of *lactobacillus spp* and incubated anaerobically at 30°C for 48 hours . After incubation cell free filtrate was obtained by centrifuging the bacterial culture at 6000 rpm for 15 minute and sterilized by filtration through 0.2mm pore size filter [(11)] .

Inhibitory effect of LAB against pathogenic bacteria *In vitro*

The antimicrobial activity of LAB isolate (cell free filtrate) on *(Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*) supplied by Dept. of Biology/ College of Science/ University of Mosul, was performed by the well diffusion method. The pathogenic tested bacteria were incubated in Brain heart Infusion (BHI) broth at appropriate temperature for 24 hours. Petri dishes containing of Muller Hinton agar were streaked previously with tested pathogenic bacteria broth culture, then put in refrigerator to solidify media, the

the activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm [11] .

In Nigeria, strain of LAB isolated from cow milk samples was tested to inhibit growth of some pathogenic bacteria by the same procedure and the results indicated the inhibitory effects on *E. coli* and *Pseudomonas aeruginosa* but not for *Bacillus cereus*, *Klebsiella pneumonia*e and *Staphylococcus aureus* [15]

Many studies were carried out using LAB isolated from poultry meat to study its antimicrobial activity on several microorganisms. The results showed that LAB inhibited *Staph. aureus*, *E.coli*, *Pseudomonas aerouginosa* with the exception of *Candida albicans* and *Proteus vulgaris* [12] .

The LAB have the ability to produce a variety of antimicrobial substances as a natural competitive means to overcome other microorganisms sharing the same niche, defined as extracellulary producted primary or modified products of bacterial ribosomal synthesis, which can have a relatively narrow spectrum of bactericidal activity[16]. LAB isolated from meat are probably the best candidates for improving the microbiological safety of these foods and act as a barrier to inhibit spoilage and /or growth of pathogenic bacteria and the biopreservation techniques for meats is in progress[17] . The Inhibition of variety of bacteria by LAB is due to a combination of many factors such as production of lactic acid, formic acid and other fermented products which reduce the pH of the meat[12] as well as other inhibitory substances such as bacteriocins, hydrogen peroxide and diacetyl production that are responsible for its most antimicrobial activity[18].

dishes were stored for 2 hours in a refrigerator. Wells were made and 100 µl of cell-free filtrate put in each well. Petri dishes were inoculated at 37°C for 24 hours, then the diameter of the inhibition zone around each well was measured with calipers in mm. [11,13].

Results

Lactic Acid Bacteria was isolated from minced meat on MRS media depending on their positive gram staining, spore forming, rod shape and motility[14]. LAB were negative for indol and catalase test. The antimicrobial activity of LAB isolates were tested on some pathogenic bacteria were summarized by using agar well diffusion assay table (1), Figure (1) showed inhibition zones of the pathogenic bacteria under study .

Table (1) : Inhibitory effect of cell free filtrate of *Lactobacillus* spp. on some pathogenic bacteria measured by using agar well diffusion method

Pathogenic bacteria	Zone diameter (mm)
B. cereus	4 mm
Proteus spp.	2.8 mm
Staph. aureus	2.5 mm
Pseudomonas aeruginosa	2 mm
E. coli	1.8 mm
Streptococcus pneumoniae	1.8 mm
Klebsiella pneumoniae	1 mm
Salmonella spp.	0.75 mm
Corynebacterium spp.	0.6 mm

Discussions

As the results indicate, the diameters of the inhibition zones were varied it ranged between 0.6 to 4 mm. This revealed that the LAB inhibited all the pathogenic bacteria tested according to [13] whose mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Similar study was carried out in Morocco by Kalalou whose studied

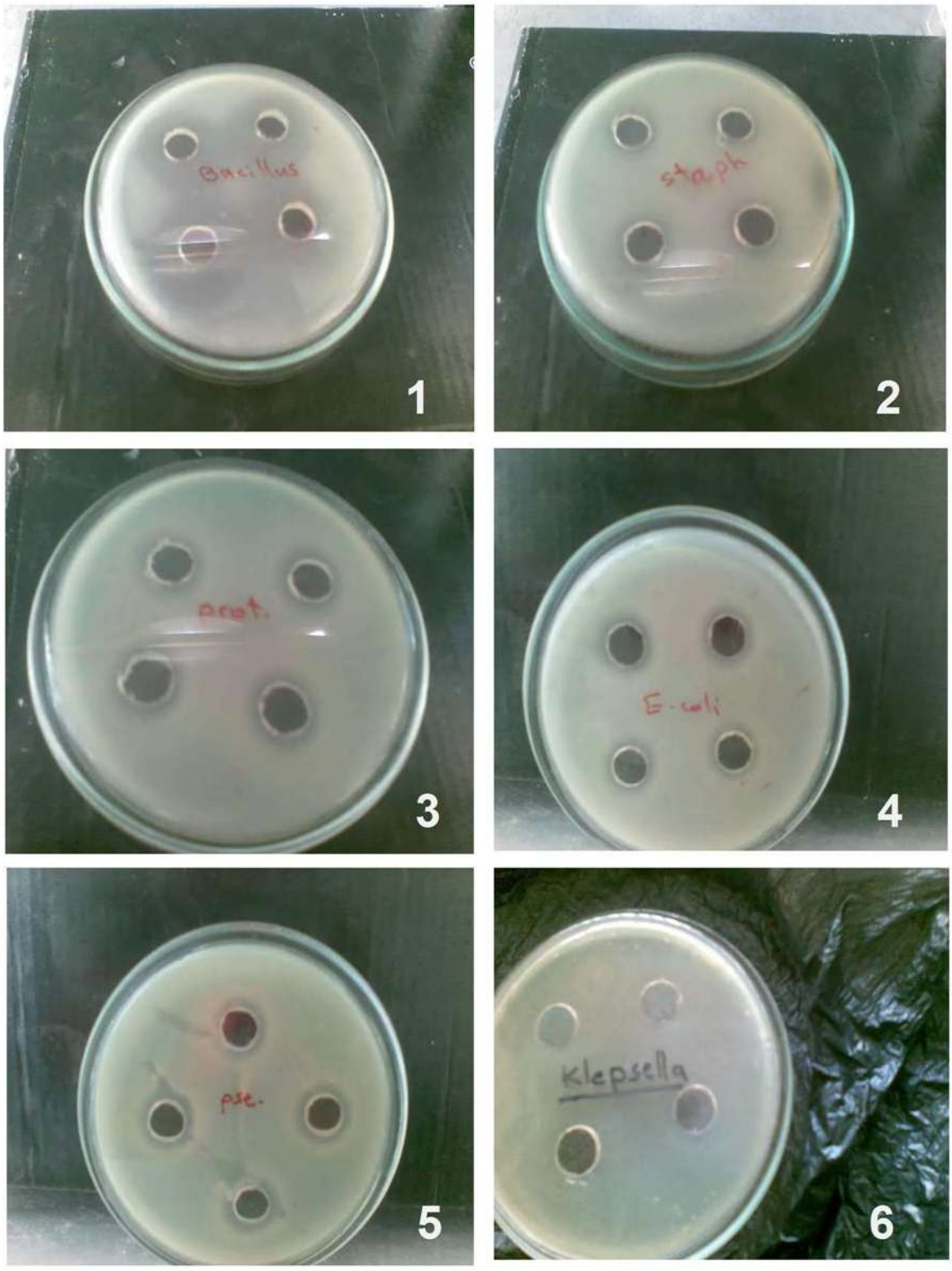

Fig (1) : Antibacterial activity of LAB on the pathogenic bacteria

1. *Bacillus cereus*
2. *Staph. aureus*
3. *Proteus* spp.
4. *E.coli*
5. *Pseudomonas aeruginosa*
6. *Klebsiella pneumoniae*

References

1. Hassan, El-S. Ferage, M. and Nahla, T. (2006) . Lactic acid and pH as indication for bacterial spoilage of meat and some meat products . J. Applied Sciences Research. 2 : 522-528.
2. Kim, JW. and Rajagopal, SN. (2001). Antimicrobial activities of *Lactobacillus crispatus* ATCC 33820 and *Lactobacillus gasseri* ATCC 33323. J. Microbiology . 39 : 146-148 .
3. Hammes, W. and Tichaczek, P. (1994) .The potential of lactic acid bacteria for the production of safe and wholesome food. J. Lebensm Unters Forsch . 198 :193-201
4. Zhu, W., Liu, W. and Wu, D. (2000) . Isolation and characterization of a new bacteriocin from *Lactobacillus gasseri* KT7. J. Applied Microbiology,. 88 : 877-886
5. Stiles, M. , (1996). Biopreservation by lactic acid bacteria . Antonie van Leeuwenhoek, 70, 331-345 .
6. Olivera, BP., Afonso, deL. and Gloria, MA. (2008). Screening of lactic acid bacteria from packaged beef for antimicrobial activity . 39 : 3680374 .
7. Lucke, F-K . (2000) . Utilization of microbes to process and preserve meat . J. Meat Science. 56: 105-115.
8. Bromberg, R., Moreno, I., Oliveira, J. (2004). Isolation of bacteriocin producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. Brazilian J. of Microbiology . 35 :137-144 .
9. Babji, Y. and Murthy, TRK. (2000). Effect of inoculation of mesophilic lactic acid bacteria on microbial and sensory changes of minced goat meat during storage under vacuum and subsequent aerobic storage. J. Meat Science. 54 :197-202 .
10. Sakhare, PZ. and Narasimha, D. (2003). Microbial profile during lactic fermentation of meat by combined starter cultures at high temperatures . J. Food Control.14: 1-5.
11. Kalalou, I., Faid, M. and Ahami, AT. (2004). Extending shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus dlbrueckii* subsp. *Delbrueckii*. 7 : 1-6 .
12. Adeskan, A., Odetoyinbo, B. and Olubamiwa, AO. (2008). Biopreservative activity of lactic acid bacteria on suya produced from poultry meat. African J. of Biotechnology. 7: 3799-3808 .
13. Schillinger, U., and Lucke, F. (1989). Antimicrobial activity of *Lactobacillus* sake isolated from meat. J. Applied and Environmental Microbiology. 55: 1901-1906.
14. Axelsson, L. (1998). Lactic acid bacteria: classification, physiology and functional aspects. 2nd ed: Marcel dekker Inc , New York. pp. 1-72 .
15. Olanrewaju, O . (2007). Antagonistic effect of *Lactobacillus* isolates from Kunnu and Cowmilk on selected pathogenic microorganisms . 9 : 63-66 .
16. Caplica, E., Fitzgerald, G. (1999). Food fermintations: role of microorganisms in food production and preservation. Int. J. Food Microbiol. 50: 131-149 .
17. Schillinge, U. and Lucke, F. (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. J. Applied and Environmental Microbiology. 55 : 1901-1906 .
18. Ogunbanwo, ST. (2005). Functional properties of lactic acid bacterial isolated from ogi and fufu, Two Nigerian fermented foods. J. food science. 27:14-21.

الفعالية الضدميكروبية لجرثومة *Lactobacillus* المعزولة من لحم البقر المثروم على بعض الجراثيم المرضية

مي عبد الحافظ عبد القادر العلاف[1] ، أميرة محمود محمد الراوي[2] ، آلاء طه المولى[2]

[1] *مركز بحوث البيئة والسيطرة على التلوث ، جامعة الموصل ، الموصل ، العراق*

[2] *قسم علوم الحياة ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق*

(تاريخ الاستلام: ١١ / ١٠ / ٢٠٠٩ ---- **تاريخ القبول:** ٢٧ / ٤ / ٢٠١٠ **)**

الملخص

يهدف البحث إلى دراسة تأثير الفعالية الضدميكروبية لجرثومة (Lactic Acid Bacteria (LAB لتثبيط نمو بعض الجراثيم الموجبة والسالبة لصبغة كرام والتي لها علاقة مع تلوث اللحوم. لذا فقد تم عزل وتشخيص الجرثومة من لحم البقر المثروم باستخدام وسط de Man Rogosa and Sharpe agar (MRS) . وقد تم ملاحظة الفعالية الضدميكروبية باستخدام طريقة الانتشار للحفر بالا كار وباستخدام وسط (Hinton Agar Muller) للجراثيم الموجبة والسالبة لصبغة كرام التالية :

(*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*).

أظهرت النتائج أن جرثومة (LAB) تمكنت من تكوين مناطق تثبيط مختلفة حول الحفر الحاوية عليها حيث كان أعلى قطر تثبيط (٤ ملم) لبكتريا *Bacillus cereus* ثم بكتريا *Proteus spp.* ، *Staph. aureus* ، *Pseudomonas aerugenosa* ، *Streptococcus pneumonia* ، *E. coli* ، *Klebsiella pneumonia* ، *Salmonella* spp. ، *Corynebacterium* spp. والتي كانت (2.8، 2.5 ، 2 ، 1.8 ، 1.8 ، 1 ، o.75 ، 0.6 ملم) على التوالي بعد تحضينها لمدة ٢٤ ساعة .